SELECTED PAPERS FROM

Physiological Regulating Medicine

INTERNATIONAL JOURNAL OF INTEGRATIVE AND BIO-REGULATORY MEDICINE

L. MILANI

GUNA COLLAGEN MEDICAL DEVICES 10 YEARS ON

– A REASONED ANALYSIS OF 2 RECENT IMPORTANT STUDIES AND LITERATURE UPDATE

NUOVA IPSA EDITORE

UP TO DATE

L. Milani

GUNA COLLAGEN MEDICAL DEVICES 10 YEARS ON

– A REASONED ANALYSIS OF 2 RECENT IMPORTANT STUDIES AND LITERATURE UPDATE

THE INTELLIGENT TRIPLE HELIX

Collagen **(COL)** is the most common structural protein biopolymer in the connective tissues of animals; in humans, it represents 25-30% of all proteins (Schmidt & Burkhardt, 2001) and 6% of total mass (Wu, 2011).

– This fibrous protein, which gives the macroscopic and microscopic anatomical structures high mechanical resilience (traction), incompressibility and tensile strength, is prevalent in the skin and subcutaneous tissues, tendons, joint capsules, ligaments, cartilage and bone.

In striated muscle, COL constitutes the main component of the endomysium (Light & Champion, 1984), the layer of areolar connective tissue that sheathes each individual muscle fibre, composed primarily by types I and II COL (Fratzl, 2008; Saladin, 2012).

– The function of COL, in addition to structuring, supporting and stabilising the somatic scaffold, is, unexpectedly, antioxidant, as demonstrated in vitro; adding COL to culture cells increases the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and phospholipid hydroperoxide glutathione peroxidase (GSH-Px) (Song et al., 2017), thereby preventing cell membrane damage caused by the ROS – reactive oxygen species (Alemán et al., 2011; Nakchum et al., 2016).

Collagen.
– Glass and steel sculpture by Julian Voss-Andreae - 2013. Rutgers University, Center for Integrative Proteomics Research. Piscataway - New Jersey, USA.

Type I COL alone accounts for ≈ **90%** of all COL in vertebrates; so far, 29 types of COL have been identified (Söderhäll et al., 2007), according to their different composition, produced by fibroblasts; mesenchymal, epithelial and endothelial cells; chondroblasts; osteoblasts and odontoblasts (Hand & Ten Cate, 2006; Gartner & Hiatt, 2007; Shoulders & Raines, 2009).

– The synthesis of mature COL involves 7 intra- and extra-cellular

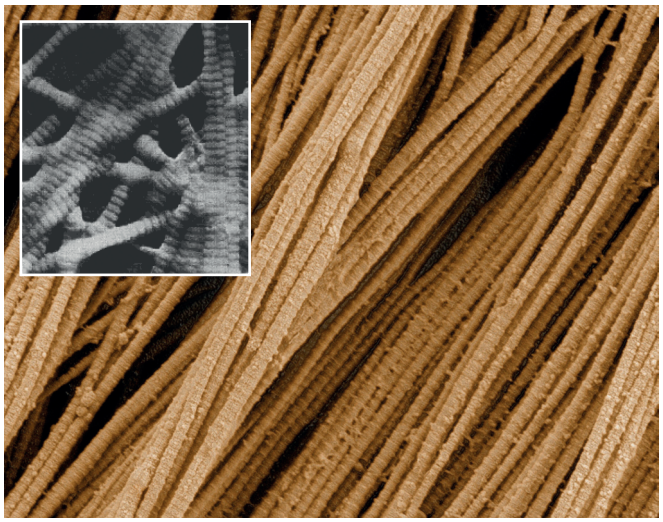


FIG. 1

Bundling up and alignment of mature COL fibres.
In the box: TEM enlargement 150,000x of some COL fibres showing the characteristic alternation of light and dark bands due to the sliding of ¼ of the lower fibre in relation the upper fibre. After 4 consecutive skiddings, the 5th fibril is aligned to the 1st one.

– In humans, starting from the sixth decade of life, this compact structure starts deteriorating, thus resulting in fibre impoverishment.

steps, from pre-procollagen (2 steps) to procollagen (3 steps) to tropocollagen (1 step) to collagen (1 step).

This complex and highly structured chain of events, which has been perfected over millions of years of evolution, can be interrupted or altered, causing, in humans, serious genetic diseases such as Ehlers-Danlos syndrome (abnormal positioning and spacing of the COL fibrils), Marfan syndrome (abnormal fibrillin 1 production), Alport syndrome (types I and IV COL synthesis defect), Osteogenesis imperfecta (type I COL synthesis defect); autoimmune diseases, such as SLE and systemic scleroderma and acquired diseases such as scurvy: many sailors of the past, in their voyages across the oceans, have paid with their life a dietary deficiency of vitamin C (L-ascorbic acid), which is absolutely necessary for the synthesis – via proline – of hydroxyproline and – via lysine – of hydroxylysine: their tropocollagen was unable to come together in fibrils.

– Like all other ancient primitive molecules, COL bears witness to the fatal causal chain.

Its right-handed **triple helix** form (the individual non-coaxial left-handed helical chains come together, interweaving with one another, due to weak chemical bonds) (Ramachandran, 1955 in Bhattacharjee, 2005), its constitutional simplicity (repeated triplets of just 5 amino acids positioned in different ways), its biochemical immutability (the structure of COL is practically identical in all species, from invertebrates to man) and its structural and functional intelligence are guarantors of flawless function. In addition to composing tendons, bone, etc., COL enters by phylogenetic right also into the composition of the extra- and intra-cellular matrix and cytoskeleton (Tomasek et al., 1982; Qin et al., 2018).

– Nature loves schemes and modules, and repeats them as often as it can and whenever they are necessary.

Synthetic glucocorticoids, especially when fluorinated, despite guaranteeing high anti-inflammatory activity, slow down the synthesis of COL in vivo and in cell cultures (Cutroneo et al., 1981).
– Paradoxically, the class of drugs most commonly prescribed in Collagenopathies actually weakens the neosynthesis of COL, impairing its efficacy over time, thereby creating a vicious therapeutic loop that becomes increasingly difficult to overcome successfully.

• In humans, COL biosynthesis peaks between **40** and **60 years** of age (collagen plateau) (in Heine, 2009) (FIG. 1); it diminishes rapidly in the sixth decade of life, together with the synthesis of elastin and the matrix proteoglycans (the total collagen pool is halved between 60 and 80 years of age).
– This reduction is governed by the late-expression age-dependent genes that encode for collagenase [matrix metalloproteinases (peptidase) = **MMPs** (previously known as matrixine)] that prevail over the **TIMPs** (tissue inhibitors of metalloproteinases), MMP-inhibiting glycoproteins (Brew et al., 2000) (FIG. 2).

The TIMPs, natural inhibitors of the MMPs and of the disintegrin-metalloproteinases (Brew & Nagase, 2010), have an anti-apoptotic function and are encoded by allocated genes on the X chromosome.
– This likely partly explains the greater longevity of female (XX allosomes) vs males (Aviv et al., 2005).
ACTH (Reichenstein et al., 2004), IL-10 (Lacraz et al., 1995), and IL-6 (Lotz & Guerne, 1991) stimulate TIMP-1, through the inhibition of the MMPs.

• It is important to remember that IL-6 plays a dual pro-inflammatory and anti-inflammatory role depending on the physiological or pathological context and the tissue it acts in.
– IL-6 modulates the destruction of COL by the MMPs; its increase in the acute phase of inflammation prevents it from spreading within the affected tissue, restricting it by the hold/staying power of the local COL.

In COL remodelling, the discrepancy by which the degradation phenomena are not adequately compensated by synthesis leads, **after ≈ 60 years of age**, to a considerable loss of the structures primarily composed of COL with a consequent anatomical and functional weakening (in particular, chrono-ageing and musculoskeletal disorders).

– Ageing is an expression of **physiological** low-grade systemic chronic inflammation (inflammageing).

The protein chains that constitute COL are massive and difficult to "create" in a laboratory: so far, it has not been possible to produce them synthetically because of their post-translational modifications (Tanrikulu et al., 2016), due to problems of symmetry (Schmitt et al., 2009) and stability (Fields, 2010). COL for therapeutic use must necessarily be extracted from animal tissues.

COLLAGEN PER OS? – A FEW WELL-FOUNDED DOUBTS

Although it has been reported that:
1) Supplementation with oral hydrolysed COL for 8 weeks improves COL density and skin moisture (Proksh et al., 2014; Choi et al., 2014; Inoue et al., 2016), and that this effect persists for 3 months (Asserin et al., 2015);
2) Patients with pressure sores treated with oral hydrolysed COL for 8 weeks obtain results superior to placebo in reducing the size of the sores (Lee et al., 2006),
these data [1) and 2)] have not been adequately confirmed and in both cases only refer to studies on structures of the integumentary system.

– The evidence is similar for the musculoskeletal system:
1) One comparative review analysed the data presented in publications including the key words: collagen, osteoarthritis, cartilage, chondrocytes (Bello & Oesser, 2006). The critical analysis of these papers revealed that oral COL does not statistically increase the synthesis of chondrocytes compared to placebo;
2) A six-month trial on 200 patients with joint pain treated with oral hydrolysed COL (1200 mg/day) shows its non-superiority to placebo. It is interesting to note that the investigators considered a mere 20% improvement in symptoms to be efficacious! (Bruyère et al., 2012);
3) A randomised, placebo-controlled prospective study conducted at Pennsylvania State University analysed the effects of hydrolysed COL in 147 athletes with joint pain (Clark et al., 2008).
Although some results were obtained at rest and when running and better performance was seen in weightlifting, the authors concluded that the follow-up was too short;
4) A study comparing the efficacy and safety of type II COL and glycosamine + chondroitin in the treatment of knee osteoarthritis in 50 subjects showed that oral type II COL is superior to glycosamine + chondroitin; however, the difference was of little clinical relevance (14% reduction in symptoms) (Crowley et al., 2009);
5) A meta-analysis on the therapeutic use of hydrolysed COL (Van Vijven et al., 2012) concluded that "the quality of the therapeutic evidence is low" and "there is insufficient evidence to recommend the generalized use of hydrolised collagen in daily practice for the treatment of patients with OA".

These results do not sufficiently support the hypothesis that the oral administration of hydrolysed COL is efficacious in improving skin condition (moisture, firmness, wrinkles), or joint pain, or, in other studies, leaky gut syndrome (Koutroubakis et al., 2003), in increasing muscle mass (Schunk et al., 2015), in strengthening nails, hair and teeth (Hexsel et al., 2017), or in certain heart diseases (Krum et al., 2011).

The dubious possibility that orally administered hydrolysed COL is actually beneficial finds its rationale in the biochemical structure of COL.
– COL is formed of long protein chains of over 1400 amino acids. The basic sequence consists of triplets of amino acids that **always** start with glycine; the 2 other amino acids are most commonly proline and hydroxyproline, and less often lysine and hydroxylysine.

• In the stomach, the gastric cells, due to the action of gastrine, secrete pepsin, which digests **any** protein or peptide introduced through the mouth, breaking it down into short chains of 5-10 amino acids that – in turn – are subsequently broken down into the single amino acids by the intestinal endo- and exo-proteases of pancreatic origin.

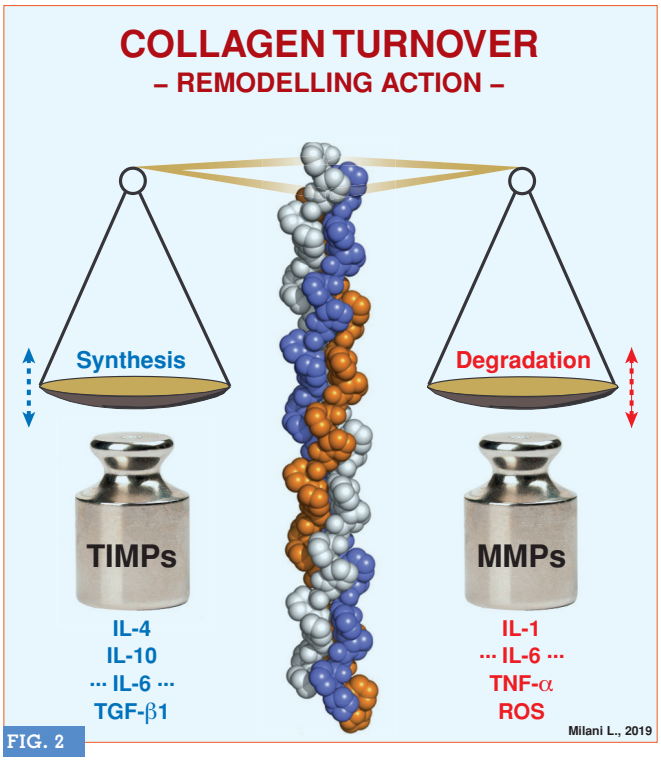


FIG. 2

The COL synthesis/degradation balance is conditioned, respectively, by anti-inflammatory cytokines and by pro-inflammatory ones, and ROS. – In normal conditions and up to ≈ 60 years of age, the two scale plates maintain a stable balance, thus guaranteeing a constant and effective COL remodelling in tissues and in extra- and intracellular matrix.

TAB. 1

Guna Collagen Medical Devices

Published articles in chronological order

CLINICAL TRIALS

PATHOLOGY	TITLE AUTHOR(S) PUBLICATION(S)	COLLAGEN MEDICAL DEVICE/S
Musculo-skeletal disorders	**Un nuovo e raffinato trattamento iniettivo delle patologie algiche dell'Apparato locomotore. – Le proprietà *bio-scaffold* del collagene e suo utilizzo clinico.** Milani L. – *La Med. Biol.*, 2010/3; 3-15. Translated into English **A new and refined injectable treatment for musculoskeletal disorders. – Bioscaffold proprieties of collagen and its clinical use.** – *Physiological Regulating Medicine,* 1/2010; 3-15.	**Description of all Guna Collagen Medical Devices**
Patello-femoral chondropathy	**Patello-femoral chondropathy treated with MD-Knee + Zeel® T trasmitted with O_2 *vs* Nimesulide + chondroitin sulphate.** Posabella G. – *Physiological Regulating Medicine*, 2011; 3-10. Translated into Italian **Terapia della condropatia femoro-rotulea con MD-Knee + Zeel® T veicolati con propulsione di O_2 *vs* Nimesulide + condroitinsolfato.** – *La Med. Biol.*, 2011/3; 3-11.	**MD-Knee**
Facial skin ageing	**Face revitalization – Biolifting with MD-Tissue.** Falconi Klein E. – *Physiological Regulating Medicine*, 2012; 15-20.	**MD-Tissue**
Low back pain	**MD-Lumbar, MD-Muscle and MD-Neural in the treatment of low back pain.** Pavelka K., Svobodová R., Jarošová H. – *Physiological Regulating Medicine*, 2012; 3-6. Translated into Italian **MD-Lumbar, MD-Muscle and MD-Neural nella terapia locale del dolore lombare.** – *La Med. Biol.*, 2012/4; 13-17.	**MD-Lumbar MD-Muscle MD-Neural**
Knee osteoarthritis	**Efficiency of collagen injections of Guna MD in patients with gonarthrosis, assessed clinically and by ultrasounds.** Nesterova R., Rashkov R., Reshkova V., Kapandjieva N. – *Physiological Regulating Medicine*, 2012; 37-39.	**MD-Knee MD-Matrix**
Vertebral disorders	**Effectiveness of integrated medicine in the control of pain in vertebral disorders: Observational Study.** Zocco R., Criscuolo S., Lorenzetti N., Senesi M. – *Physiological Regulating Medicine*, 2012; 41.	**MD-Lumbar**
Abdominal adiposity	**Acumesotherapy with Guna-Matrix in patients with localized abdominal adiposity.** Elenkova S., Pozharashka J. – *Physiological Regulating Medicine*, 2012; 43.	**MD-Matrix**
Knee osteoarthritis	**Application and assessment of efficacy of collagen injections Guna MDs in gonarthrosis.** Boshnakov D. – *Physiological Regulating Medicine*, 2013; 29-30.	**MD-Knee MD-Muscle**
Algic arthro-rheumopathies	**I Collagen Medical Devices nel trattamento locale delle artro-reumopatie algiche. – Rassegna degli studi clinici e *clinical assessment* 2010-2012.** Milani L. – *La Med. Biol.*, 2013/2; 3-18. Translated into English **The Collagen Medical Devices in the local treatment of the algic arthro-rheumopathies. – Review of the clinical studies and clinical assessment 2010-2012.** – *Physiological Regulating Medicine,* 2013, 21-36.	**Corresponding MDs**
Hip osteoarthritis	**Intra-articular administration of MD-Hip in 7 patients affected by hip osteoarthritis unresponsive to viscosupplementation. Six-month multicenter trial.** Migliore A., Massafra U., Bizzi E., Vacca F., Tormenta S. – *Physiological Regulating Medicine*, 2013; 31. Translated into Italian **Somministrazione intra-articolare di MD-Hip in 7 pazienti con osteoartrosi dell'anca non responsivi alla viscosupplementazione. - Studio multicentrico della durata di 6 mesi.** – *La Med. Biol.*, 2013/2; 13.	**MD-Hip**

PATHOLOGY	TITLE AUTHOR(S) PUBLICATION(S)	COLLAGEN MEDICAL DEVICE/S
Hip osteoarthritis	**Efficacy of injections MD-Hip and MD-Matrix in the treatment of coxarthrosis.** **– Clinical and ultrasonographic evaluation.** Tivchev P. – *Physiological Regulating Medicine*, 2013; 31-32. Translated into Italian **Efficacia delle iniezioni di MD-Hip e MD-Matrix nel trattamento della coxartrosi.** **– Valutazione clinica ed ecografica.** – *La Med. Biol.*, 2013/2; 13-14.	**MD-Hip** **MD-Matrix**
Hip osteoarthritis	**Il ruolo del Medical Device-Hip nella terapia infiltrativa ecoguidata dell'artrosi di anca.** Milano E. – *La Med. Biol.*, 2013/4; 13. Translated into English **The role of MD-Hip in ultrasound-guided injection therapy in osteoarthritis of the hip.** – *Physiological Regulating Medicine*, 2018; 3-8.	**MD-Hip**
Musculo-skeletal injuries	**La gestione biologica dell'atleta: medicinali omotossicologici e Collagen Medical Devices.** Alfieri N. – *La Med. Biol.*, 2013/4; 27-32.	**MD-Muscle** **MD-Shoulder**
Osteoarthritis	**Trattamento delle patologie articolari con Collagen Medical Devices.** **– Studio clinico su 257 pazienti.** Ottaviani M. – *La Med. Biol.*, 2014/3;11-21. Translated into English **Treatment of joint conditions with Guna Collagen Medical Devices.** **– Clinical study on 257 patients.** – *Physiological Regulating Medicine*, 2018; 2018; 18-25.	**Corresponding MDs**
Knee osteoarthritis	**A double blind randomized active-controlled clinical trial on the intra-articular use of MD-Knee versus sodium hyaluronate in patients with knee osteoarthritis ("Joint").** Martin Martin L.S., Massafra U., Bizzi E., Migliore A. – *BMC Musculoskeletal Disorders*, 2016; 17:94.	**MD-Knee**
Chronic pain	**Collagen MDs for Chronic Pain. Efficacy and tolerability in chronic treatment in 124 patients.** Guitar Vela J., Folch Ibáñez J. – *Physiological Regulating Medicine*, 2016-2017; 9-12.	**MD-Lumbar** **MD-Ischial** **MD-Muscle** **MD-Neural**
Shoulder tendinitis	**Collagen Medical Device infiltrations in shoulder pathologies. Calcific supraspinatus tendinitis.** Zurita Uroz N. – *Physiological Regulating Medicine,* 2016-2017; 15-17.	**MD-Shoulder**
Piriformis Syndrome	**3 years in Luhačovice Spa with Collagen Medical Devices injections in the treatment of Piriformis Syndrome.** Staňa J. – *Physiological Regulating Medicine*, 2016-2017; 19-20.	**MD-Matrix** **MD-Muscle**
Myofascial pain Syndrome	**MD-Muscle in the management of myofascial pain syndrome.** Alfieri N. – *Physiological Regulating Medicine*, 2016-2017; 23-24.	**MD-Muscle**
Knee osteoarthritis	**Efficacy and safety evaluation of Guna Collagen MDs injections in knee osteoarthritis.** **– A case series of 30 patients.** Reshkova V., Rashkov R., Nesterova R. – *Physiological Regulating Medicine*, 2016-2017; 27-29.	**MD-Knee** **MD-Muscle**
Hip osteoarthritis	**Intra-articular administration of MD-Hip in 24 patients affected by symptomatic hip osteoarthritis.** **– A 24-month cohort study.** Giovannangeli F., Bizzi E., Massafra U., Vacca F., Tormenta S., Migliore A. – *Physiological Regulating Medicine*, 2016-2017; 31-32.	**MD-Hip**
Rotator cuff Syndrome	**Clinical and sonographic assessment of the effectiveness of Guna Collagen MDs injections in patients with partial thickness tear of the rotator cuff.** Nesterova R., Rashkov R., Petranova T. – *Physiological Regulating Medicine*, 2016-2017; 35-37.	**MD-Shoulder** **MD- Muscle**
Knee osteoarthritis	**Usefulness of Guna Collagen Medical Devices in the treatment of knee pain.** Mariconti P. – *Physiological Regulating Medicine*, 2016-2017; 39-40.	**MD-Knee** **MD-Matrix**

PATHOLOGY	TITLE AUTHOR(S) PUBLICATION(S)	COLLAGEN MEDICAL DEVICE/S
Low back pain	**Injectable Guna Collagen Medical Device in functional recovery from sport traumatology. – Case Reports** Massullo C. – *Physiological Regulating Medicine*, 2016-2017, 3-7. Translated into Italian **I Guna Collagen Medical Device nella ripresa funzionale dopo traumi sportivi. – Case Reports** – *La Med. Biol.*, 2017/2; 45-50.	**MD-Lumbar** **MD-Matrix** **MD-Muscle**
Shoulder and knee osteo-articular pain	**Associazione di Collagen MDs e Chelt Terapia nel dolore osteo-articolare di spalla e di ginocchio.** Feninno D., Bonacina A. – *La Med. Biol.*, 2017/2; 37-42.	**MD-Shoulder** **MD-Knee**
Rhizarthrosis	**Rizartrosi e omeosiniatria. Efficacia di Zeel® T e di MD-Small Joints a confronto.** Bernardini G. – *La Med. Biol.*, 2018/2; 15-23.	**MD-Small Joints**
Low back pain	**Chronic Low Back Pain: Current Pharmacotherapeutic Therapies and a New Biological Approach.** Pavelka K., Jarosova H., Sleglova O., Svobodova R., Votavova M., Milani L., Prochazka Z., Kotlarova L., Kostiuk P., Sliva J., Meroni A.M. – *Current Medicinal Chemistry*, 2018 May 13, 25: 1-8.	**MD-Lumbar** **MD-Muscle** **MD-Neural**
Myofascial pain of masseter muscle	**Comparison between Collagen and Lidocaine Intramuscular Injections in Terms of Their Efficiency in Decreasing Myofascial Pain within Masseter Muscles: a Randomized, Single-Blind Controlled Trial.** Nitecka-Buchta A., Walczynska-Dragon K., Batko-Kapustecka J., Wieckiewicz M. – *Pain Research and Management*, 2018; 1-10.	**MD-Muscle**

PRE-CLINICAL TRIAL

COLLAGEN MEDICAL DEVICE	TITLE AUTHORS PUBLICATION	TRIAL
MD-Tissue	**Effect of Collagen-Based Compound on Morpho-Functional Properties of Cultured Human Tenocytes.** Randelli F., Menon A., Giai Via A., Mazzoleni M.G., Sciancalepore F., Brioschi M., Gagliano N. – *Cells*, 2018 Dic; (246) 7: 8-14.	In vitro trial

NOTE

- The articles published in *Physiological Regulating Medicine* and other publications are available online. Please visit: **https://collagenmd.guna.com**
- The articles published in *La Medicina Biologica* are available online. Please visit: **www.medibio.it ⟶ La Medicina Biologica**

– In the intestine, the single amino acids or di/tripeptides are carried to the liver, where they are used as is or take part in de novo protein synthesis or – if they are in excess – they are converted into fat or used for energy purposes (gluconeogenesis) (Kumar & Gill, 2018).

▶ Proline and glycine **are not** "essential" amino acids, rather they can normally be synthesised by the body; nor are they "rare" amino acids, as they are commonly present in readily available foods:
– Proline in cheese and other milk-derived products, beef, pork, chicken, fish/seafood, pulses, cereals, flour, pasta and dried fruit;
– Glycine in fish/seafood, pork, vegetables, etc.

▶ Lysine **is** an essential amino acid; but it is readily available in beef, certain fish (cod, sardines), poultry, cheese and other milk-derived products, eggs and pulses.

The age-dependent physiological COL neosynthesis deficit is not due to a deficiency of the amino acids of which COL is composed, rather to the progressive silencing of certain TIMP template genes that commences at ≈ 60 years of age.
– It is not the raw material that is lacking, but the **anabolic control** genes of COL remodelling that start to dwindle.
There is no shortage of bricks… it is the bricklayer who is absent or – if present – is unable to understand the instructions of the engineer.

JOINT HYPERMOBILITY SYNDROME

Benign (or common) joint hypermobility **(JH)** syndrome (Grahame et al., 1992; Bird, 1993), caused by **slack ligaments**,

representing the upper part of a Gaussian distribution of the normal range of joint mobility considering age, gender and ethnic background, decreases with age (Silverman et al., 1970).
Epidemiological studies have shown that JH, depending on the criteria adopted, is present in 25% of the Caucasian population (Al-Rawi et al., 1985; Birrell et al., 1994).

– Due to partial, even minor, alterations in fibrillin, which plays an important role in the aggregation of the elastic fibres, many children and adults with slack ligaments (Biro et al., 1983; Bridges et al., 1992) develop joint pain and osteoarthritis later in life (Beighton et al., 2012).
JH presents primarily in the proximal and distal interphalangeal, metacarpophalangeal and tibiotarsal joints (Grahame & Jenkies, 1972) and in the spine (prolapsed disks, spondylolisthesis).

With ageing, all the COL forming the **peri-articular** structures (ligaments, capsules, tendons, muscles) and the **intra-articular** structures (ligaments of the large joints alone) undergoes significant qualitative and quantitative changes, that make the heads of the articular bones more mobile along their planes of movement, rather than being held firmly in place.

Joint hypermobility leads to abnormal weightbearing with consequent inflammation and later degeneration of the joint cartilage, the first step towards osteoarthritic degeneration. In short: the incorrect positioning of the two adjacent heads of the bones forming a joint as established by physiological biomechanics causes **wear**, **pain** and **movement difficulties**.

There is strong evidence that JH is an important factor of the pathogenesis of osteoarthritis (Grahame, 1989; Grahame et al., 1992; Jonsson et al., 1996) caused by mechanical overuse due to a lack of the normal alignment of the heads of the adjacent bones caused by the **laxity** of the **joint capsule** and of the extra- and intra-articular **ligaments**.

• This is a crucial concept that deserves careful attention.

– It has already been dealt with in a paper by this author (Milani, 2013) which can be consulted for further definitions and details.

Although NSAIDs and DMARDs – however they are considered – can be efficacious for improving pain symptoms, they **do nothing** to improve the firm hold of a joint, its conformation or its stabilisation.

– Simply treating the symptoms without considering the original cause of a condition deprives medical practice of a fundamental component and does not deliver stable results.

GUNA COLLAGEN MEDICAL DEVICES

Since the presentation and introduction into peri- and intra-articular and soft tissue injection therapy of Guna Collagen Medical Devices (**MD**s) (Milani, 2010) various researchers have conducted on the therapeutic action of these products in the different medical conditions for which they were developed, many of which on the osteo-artro-myofascial system, **28** good-quality **clinical trials**, some of which were published in peer-reviewed, indexed international journals with high impact factors (BMC Musculoskeletal Disorders, Pain Research and Management, Current Medicinal Chemistry) (TAB. 1).

– In over half of these publications (16 out of 28) (TAB. 1), the authors consider a treatment combination of 2 or more medical devices (e.g. for chronic lower back pain: Pavelka et al., 2012, 2018).

• The salient characteristics and a reasoned analysis of two important recent studies are provided below; the first is a clinical study, the second a basic (preclinical) study on the therapeutic and biological properties of 2 Guna Collagen MDs: **MD-Muscle** and **MD-Tissue**.

Nitecka-Buchta A., Walczynska-Dragon K., Batko-Kapustecka J., Wieckiewicz M.

Comparison between Collagen and Lidocaine Intramuscular Injections in Terms of Their Efficiency in Decreasing Myofascial Pain within Masseter Muscles: A Randomized, Single-Blind Controlled Trial.

– **Pain Research and Management**, Volume **2018**, Article ID 8261090. 10 pages.
Publication: 3 June 2018.

– Authors – Operational sites
Nitecka-Buchta A., Walczynska-Dragon K., Batko-Kapustecka J.: Department of Temporomandibular Disorders, SMDZ Zabrze Unit – Medical University of Silesia, Katowice, Poland;
Wieckiewicz M.: Department of Experimental Dentistry, Faculty of Dentistry – Medical University of Wroclaw, Poland.

1) Foreword
Myofascial pain syndrome is a common disorder caused by trigger points, hard, localised, palpable nodules, that are painful on intermediate palpation. The anatomical damage is

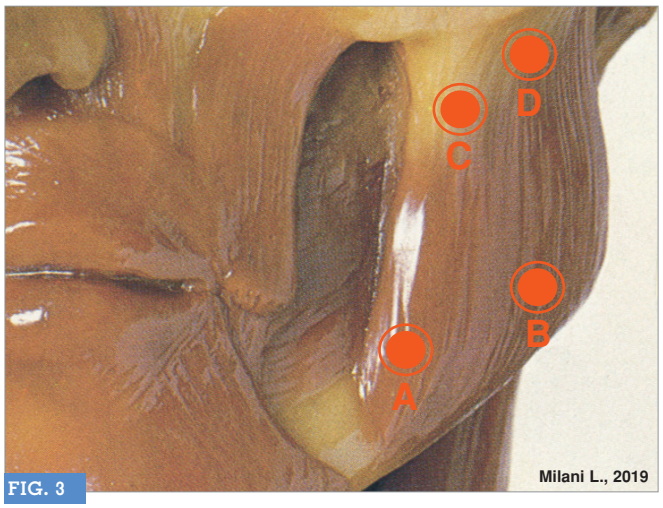


FIG. 3

Masseter muscle – Trigger Points (TPs). The TPs of masseter muscle can project pain: A – to the jaw; B – to the ear (external auditory meatus); C – to the maxilla; D – to orbital and frontal region.
– In the myofascial syndrome of the masseter muscle are generally involved 1-2 TPs, more frequently the TP at the jaw angle (B).

characterised by lesions of the myofilaments and sarcolemma. The functional damage is characterised by local hypoxia and the release of pro-inflammatory mediators (bradykinin, catecholamines, neuropeptides, cytokines) with persistent pain and inflammation. Certain close trigger points join to form areas of myogelosis, in which the level of O_2 is extremely low, leading to a reduction in ATP.
– Lidocaine hydrochloride 2% is commonly used for local injection for nerve blocks and analgesia of the superficial tissues; the mechanism of action consists in blocking the Na^+ channels of the cell membrane.
In trigger point therapy, it is used without a vasoconstrictor due to the risk of ischaemic necrosis.
The duration of the analgesic effect varies from 30 minutes to 3 hours.

2) Purpose of the study

– Assessment of the efficacy of local intramuscular injections of Collagen Medical Device Muscle **(MD-Muscle)** or **lidocaine** in reducing the pain caused by trigger points in the masseter muscle (FIG. 3).

3) Materials and methods

3-1 Participants

From a group of 102 Caucasian patients treated at the Department of Temporomandibular Disorders – Medical University of Silesia, Katowice, Poland, 50 patients who had had chronic myofascial pain (MFP) of the masseter muscle for an average of 8.5 months were identified and included in the study.

3-2 Inclusion criteria

(1) Age ≥18 and ≤80 years;
(2) Presence of myofascial pain in the masseter muscle according to Temporomandibular Disorder Diagnostic Criteria (DC/TMD) (II.1.A. 2 and 3) (Peck et al., 2014);
(3) Presence of latent or active trigger points in the masseter muscle(s) observed on palpation;
(4) Patient consent to inclusion.

3-3 Exclusion criteria

(1) Patients undergoing dental treatment; **(2)** Patients on treatment with or dependence on analgesics and/or drugs affecting muscle function; **(3)** Patients who had had head or neck injuries in the previous 2 years; **(4)** Edentulous patients and those with occlusal contacts without arch supports; **(5)** Patients treated by a neurology specialist for neurological disorders and/or neuropathic pain and/or headache; **(6)** Patients who had had radiotherapy; **(7)** Odontogenic pain; **(8)** Pregnancy or breastfeeding; **(9)** Malignancies; **(10)** Severe mental disorders; **(11)** Drug and/or alcohol addiction; **(12)** Contraindications to injection therapy; **(13)** Patients with needle phobia; **(14)** Hypersensitivity to the substances involved in the study.

The 50 patients were split into 3 Groups using a simple computerised randomisation procedure: 1) Group I – MD-Muscle = 18; 2) Group II – lidocaine = 15; 3) Group III – sterile physiological solution = 17. After randomisation, 7 patients refused to take part in the study. The 3 Groups were therefore resized **(43 patients)** as follows (TAB. 2):
– Group I – Experimental group proper – **Collagen MD-Muscle**, 2 mL = **15 patients** (5 M, 10 F), mean age 37.2 ± 4.97 years;
– Group II – Control group proper – **lidocaine 2%**, 2 mL = **13 patients** (5 M, 8 F), mean age 42.8 ± 0.98 years;
– Group III – Neutral control group – **sterile saline solution**, 2 mL = **15 patients** (7 M, 8 F), mean age 40.3 ± 1.18 years.

The 3 substances used in the trial were injected into the trigger point(s) identified [unilateral (40 patients), bilateral (3 patients)] by the same physician.

Patients were not told which substance would be injected.
– Study design consisted in 4 steps: (1) screening for study participation and enrolment; (2) baseline - first injection; (3) 1st follow-up and second injection; (4) 2nd follow-up.

TAB. 2

Baseline characteristics of 43 patients with MFP within masseter muscles included in the study.

	Group I	Group II	Group III
Male/female, *n*	5/10	5/8	7/8
Age (years)	37.2 ± 4.97	42.8 ± 0.98	40.3 ± 1.18
Duration of myofascial pain (weeks), mean (SD)	30.2 ± 31.48	34.3 ± 29.26	38.3 ± 26.47
Bilateral involvement of myofascial pain (number of patients)	2	1	0

The period elapsing between (2), (3) and (4) was one week **(days 0, 7** and **14)**.

4) Measurement of treatment outcomes
– Visual-analogue scale (VAS 1-10) - days 0, 7 and 14.
Primary outcome.
– Superficial electromyography (EMGs) - days 0, 7 and 14.
Secondary outcome.

4-1 VAS
The average reduction in pain intensity at days 7 and 14 was (FIG. 4, TAB. 3):
– Group I – MD-Muscle = -4.3 **(-53.75%)**.
From 8 (baseline) to 4.6 (day 7) to 3.7 (day 14).
– Group II – lidocaine 2% = -2.3 **(-25%)**.
From 8.3 (baseline) to 7.4 (day 7) to 6 (day 14).
– Group III – sterile saline solution = -1.63 **(-20.1%)**.
From 8.13 (baseline) to 6.8 (day 7) to 6.5 (day 14).

Comments by the author
A) It can be observed that, unlike lidocaine, MD-Muscle was very efficacious even just 7 days after the first injection, with a **40%** reduction in pain symptoms, which dropped further after the second injection.
B) The similarities in the results between lidocaine and sterile saline solution at both 7 and 14 days should come as no surprise, as lidocaine produces an analgesic effect that wears off within 3 hours from the injection.
At day 14, the difference in VAS score was -2.3 for lidocaine and -1.63 for sterile saline solution.
C) Similarly, it should come as no surprise that sterile saline solution produces a modest analgesic effect when injected into a myofascial trigger point: the analgesic effect – in this case – is not due to the sterile saline solution in itself, rather to its mechanical effect and the stimulation of the Aδ and c nerve fibres that introducing a needle into a trigger point produces (Milani, 2003, 2004).
D) MD-Muscle is more than **twice** as efficacious as lidocaine 2% (-53.75% vs -25.0%).

4-2 EMGs
The mean reduction in the voltages recorded on days 7 and 14 was (FIG. 5, TAB. 4):
– Group I – MD-Muscle = -32.9 μV **(-59.2%)**.
From 56.6 μV (baseline) to 32.6 μV (day 7) to 23.7 μV (day 14).
– Group II – lidocaine 2% = -23.5 μV **(-39.3%)**.
From 59.9 μV (baseline) to 42.4 μV (day 7) to 36.4 μV (day 14).
– Group III – sterile saline solution = -8.9 μV **(-14%)**.
From 64.1 μV (baseline) to 60.2 μV (day 7) to 55.2 μV (day 14).

Comments by the author
A) The EMGs μV values given are the average values of 3 measurements.
B) The % differences for the EMGs values in Groups I and II are consistent with the VAS values for the same Groups (see above).

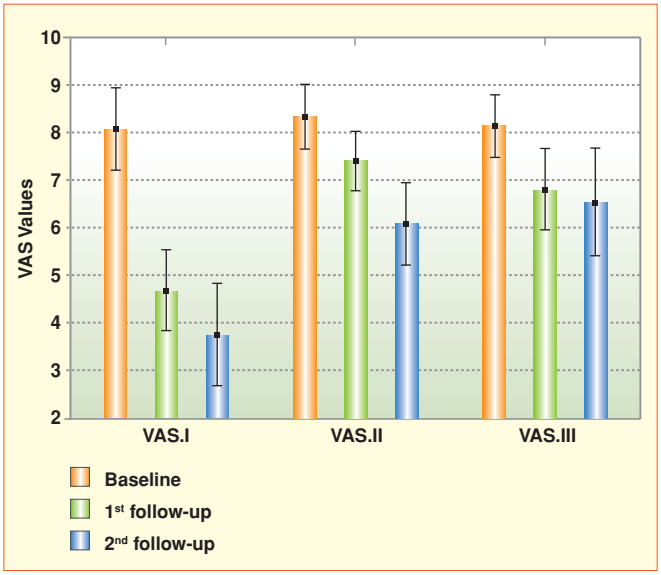


FIG. 4

VAS mean value changes in Group I, Group II, and Group III during the trial (days 0, 7, and 14).

Visit	Group I	Group II	Group III
Baseline	8	8.3	8.13
1st follow-up visit	4.6	7.4	6.8
2nd follow-up visit	3.7	6	6.5
VAS changes	**– 4.3**	**– 2.3**	**– 1.63**
Percentage VAS changes	**– 53.75%**	**– 25%**	**– 20.1%**

TAB. 3

Changes in VAS mean values in Group I, Group II, and Group III after 14 days.

C) In the study, the EMGs values for the same muscle on the asymptomatic side were also recorded in 40 patients [data shown in TAB. 4 (**NP**)].

5) Side effects
Approximately 30 minutes after the injection of MD-Muscle in the trigger point(s) identified in the masseter muscle(s), the patients reported an ache when opening their mouth, a slight local swelling feeling and mild local muscle stiffness. After about 1 hour, all these symptoms had disappeared.
In 9 patients out of 43 (21%), small bruises appeared at the needle introduction point(s) after the injection.
All these effects were temporary and completely reversible.
No severe adverse effects occurred during the study.

6) Discussion
The injections of MD-Muscle, lidocaine and sterile saline solution into the trigger point(s) of the masseter muscle(s) in the treatment to reduce myofascial pain at the same points had different results in the 3 Groups.

– The **best results** were obtained in **Group I – MD-Muscle**: better antinociceptive results (VAS reduction -4.3 = **-53.75%**)

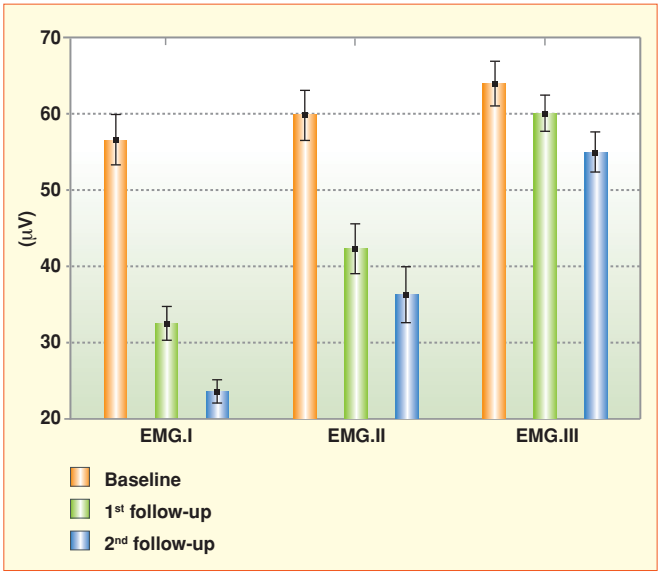


FIG. 5

Changes in mean values of superficial electromyographic activity of masseter muscles in Group I, Group II, and Group III during the trial (days 0, 7, and 14).

Visit	Group I (μV)	Group II (μV)	Group III (μV)
Pain side			
Baseline	56.6	59.9	64.1
1st follow-up visit	32.6	42.4	60.2
2nd follow-up visit	23.7	36.4	55.2
EMG changes	**– 32.9**	**– 23.5**	**– 8.9**
Percentage EMG changes	**– 59.2 %**	**– 39.3 %**	**– 14 %**
No pain side			
Baseline	34.3	38.7	36.6
1st follow-up visit	34.6	39.2	34
2nd follow-up visit	35.2	37.7	36.5
EMG changes	**+ 0.9**	**– 1**	**– 0.1**
Percentage EMG changes	**+ 2.6 %**	**– 2.5 %**	**– 0.3 %**

TAB. 4

Changes in EMG mean values in Group I, Group II, and Group III after 14 days.

and reduction in EMGs values (-32.9 μV = **-59.2%**).

Authors' conclusions

"The study showed that the intramuscular injection of MD-Muscle in the masseter muscle is more efficacious that the intramuscular injection of lidocaine".

References cited in the original publication
– Peck C.C., Goulet J.P., Lobbezoo F. et Al. – Expanding the taxonomy of the diagnostic criteria for temporomandibular disorders. Journal of Oral Rehabilitation, vol. 41, no. 1, pp. 2-23; **2014**.

Randelli F., Menon A., Giai Via A., Mazzoleni M., Sciancalepore F., Brioschi M., Gagliano N.

Effect of a Collagen-Based Compound on Morpho-Functional Properties of Cultured Human Tenocytes.

– **Cells**, **2018**, 7, 246; doi: 10.3390/cells7120246.
14 pages.
Publication: 6 December 2018.

– Authors - Operational sites
Randelli F., Giai Via A., Mazzoleni M., Brioschi M.: Centro di Chirurgia dell'Anca e Traumatologia [Hip surgery & Trauma Centre] - I.R.C.C.S Policlinico San Donato (San Donato Milanese, Milan - Italy);
Menon A., Sciancalepore F.: Azienda Socio Sanitaria Territoriale [Local Public Health Authority] Centro Specialistico Ortopedico Traumatologico Gaetano Pini-CTO [Gaetano Pini-CTO Specialised Orthopaedic Trauma Centre], 1a Clinica Ortopedica [1st Orthopaedic Clinic], Milan - Italy;
Gagliano N.: Dipartimento di Scienze Biomediche per la Salute [Department of Biomedical Sciences for Health] – Università degli Studi di Milano, Milan - Italy.

The study was presented at:
– XXIX A.M.I.O.T. National Congress, Bologna, 30 June 2018;
– 72nd Congress of the Italian Anatomy and Histology Society. Parma, 20-22 September 2018;
– World Congress of the International Society for Hip Arthroscopy (ISHA). Melbourne, Australia, 4-6 October 2018.

1) Foreword

The tissues of the osteo-arthro-myofascial system, in particular the soft tissues and, of these, especially the joint capsules, ligaments and tendons, throughout life are subject to functional overload, accidents and ageing → inflammation → pain → **degenerative evolution** and consequent **functional alterations**.

2) Collagen - Metabolism

The physiological turnover of COL requires that its degradation by the matrix metalloproteinases **(MMPs)** is regulated by the tissue inhibitors of metalloproteinases **(TIMPs)**.
– This physiological homeostatic regulation allows the body to have effective new COL at its disposal at all times, therefore biosynthesis must prevail slightly over biodegradation. When the action of the MMPs prevails over that of the TIMPs, as occurs physiologically from the age of ≈ 60 years, there is an evolution of the pathological situation towards tissue degradation. The MMPs are controlled by pro-inflammatory cytokines and by the ROS (reactive oxygen species), the TIMPs are regulated by anti-inflammatory cytokines (FIG. 2).

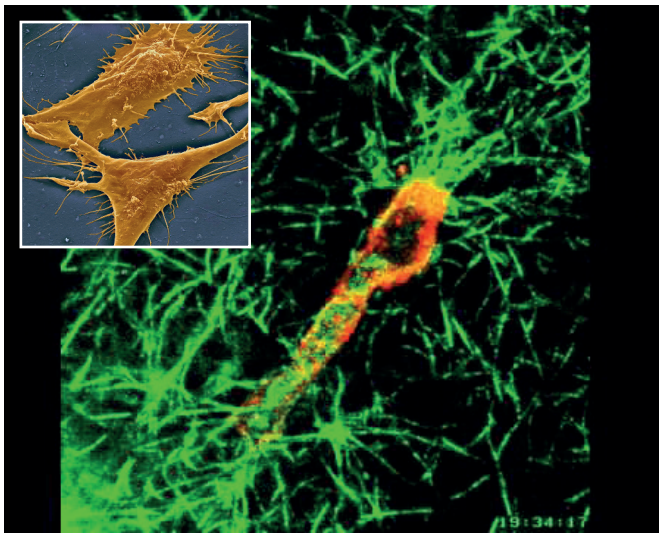


FIG. 6

Fibroblast and COL fibres (green).
– Depending on the anatomical structure that will host the newly formed COL fibres, these can be arranged, without an apparent predetermined order, as in the extracellular matrix or according to precise alignments, as in muscle fascia, ligaments and tendons.
– In the articular capsules the COL fibres tend to get arranged in a net-like pattern, reinforcing and stabilizing the joint.

It is, once again, the good "equilibrium" of the Th1/Th2 cytotypes of the immune balance that regulates the synthesis and degradation of COL.

3) Tendons - Histology

The tendons are composed primarily by
1) type 1 COL fibres; **2)** extracellular matrix rich in glycosaminoglycans (GAGs) and in proteoglycans (PGs).
– The special structure of COL provides the tendon with tensile strength, whereas the extracellular matrix provides the COL fibres with a scaffold.
Cell elements are observed between the COL fibres: the **tenocytes** (fibrocytes with a triangular or – more often – elongated and jagged appearance), promotors of the synthesis of matrix and pre-procollagen (FIG. 6).
COL assembles first to form tropocollagen, which comes together to form fibrils, fibres, 1st, 2nd and 3rd order fascicles and, eventually, the tendon.
COL is synthesised inside the tenocytes and assembled outside of them, thereby making it available to the tissues.

4) Purpose of the study

In vitro analysis of the effects of **MD-Tissue** on human tenocytes, in order to investigate and understand the molecular mechanisms underlying the action of this Medical Device and – therefore – **how** it is able to affect tendon homeostasis and repair.

5) Experimental cytotype

Cultures of differentiated **human tenocytes** obtained from biopsy specimens of heathy tendons of the gluteus minimus muscle taken from 8 volunteer patients (4 M, 4 F; age 64.8 ± 7.2 years), undergoing hip replacement surgery.
The tenocytes were cultivated:
A) on plates containing a thin coating of MD-Tissue, or
B) on plates **not** containing MD-Tissue [no coating **(NC)** control].

6) Parameters investigated

6-1 Cell proliferation (FIG. 7)

The tenocytes cultivated on MD-Tissue proliferate (number of tenocytes/24 h), after 72 hours of incubation: tenocytes + MD-Tissue = from 100 to 200 **(+100%)**; tenocytes without MD-Tissue = from 100 to 150 **(+50%)**.

6-2 Gene expression - real-time PCR x lysyl hydroxylase (FIG. 8)

The levels of messenger-RNA (mRNA) for lysyl hydroxylase (LH2b) are not influenced by MD-Tissue at any of the time points considered (24 h, 48 h, 72 h).

6-3 Protein analysis - Slot Blot neosynthesis of type I COL (FIG. 9)

The neosynthesis of type I COL is **higher** in tenocytes cultivated on MD-Tissue than in the NC controls.

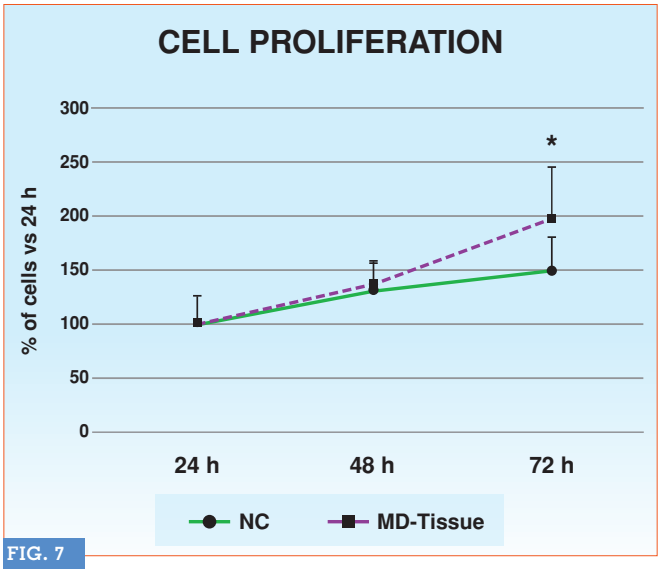


FIG. 7

Growth curves of tenocytes grown without (NC) or on MD-Tissue at the indicated time points. Data are expressed as percentages vs the time point T24 and are mean + SD. * *p* <0.05 vs 72 h NC.

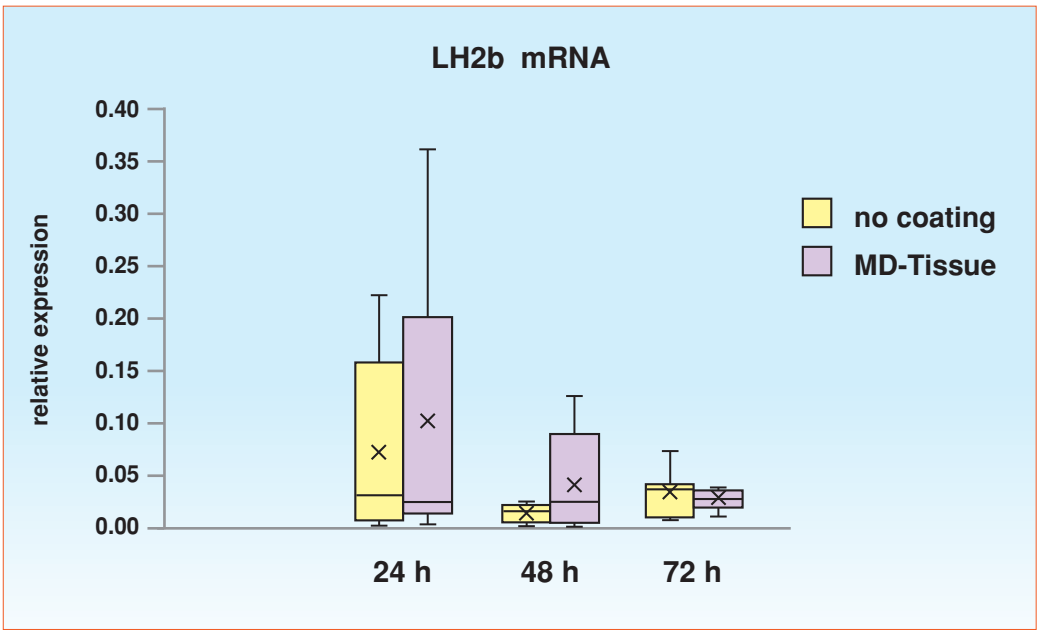


FIG. 8

Bar graphs showing mRNA levels for Long lysyl hydroxylase 2 (LH2b) in NC and MDTissue tenocytes (COL) assessed by real-time PCR. PCR - *Polymerase Chain Reaction*.
– Data were normalized on GAPDH *(GlycerAldehyde 3-Phosp. DeHydrogenase)* **gene expression and are expressed as mean ± SD for at least 2 independent experiments for the 8 samples run in duplicate. X = mean.**

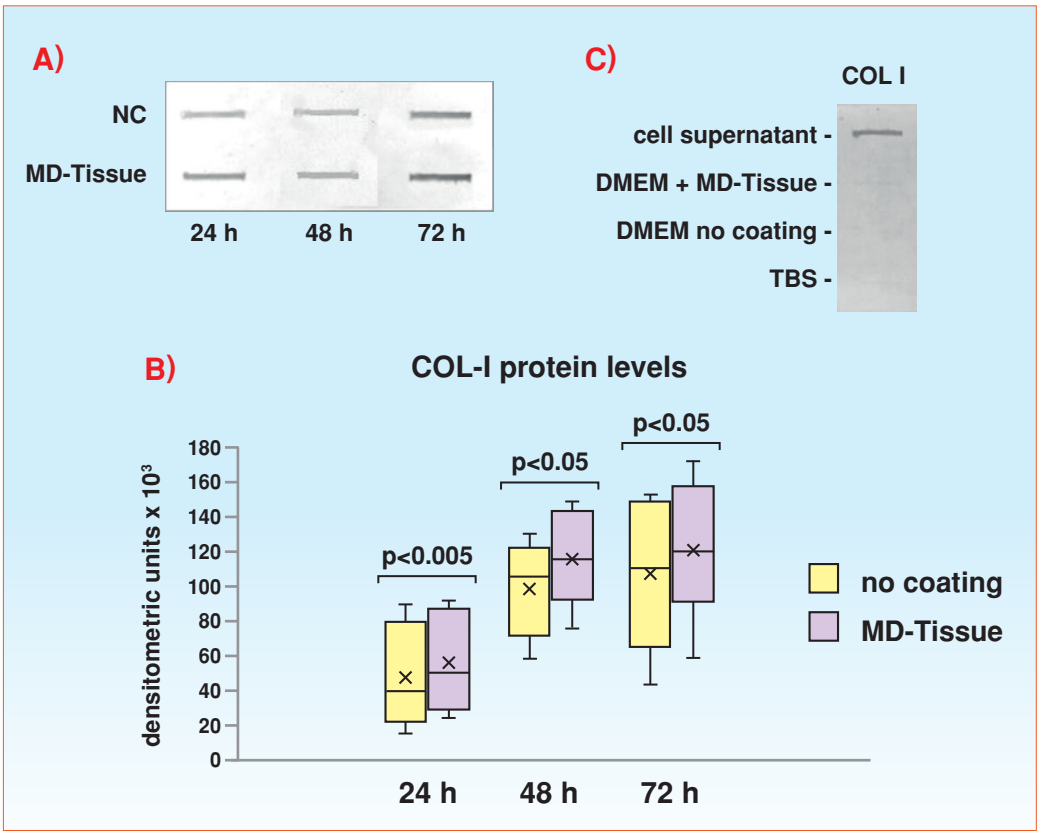


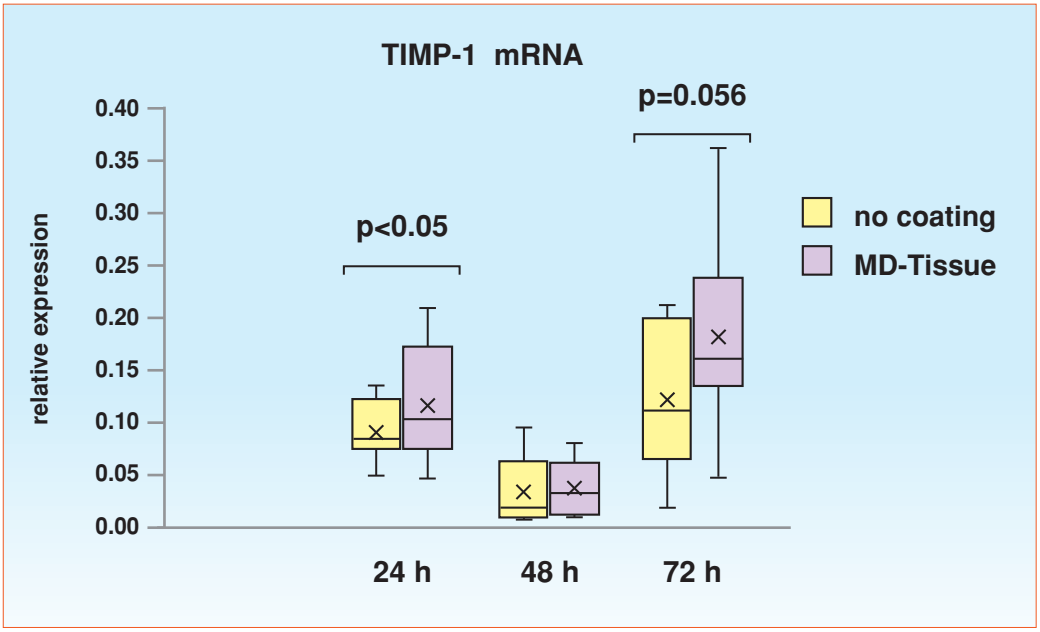
FIG. 9

Representative slot blot analysis for collagen type I (COL-I) expression (A) expression in cell-culture medium of tenocytes cultured without coating (NC) or on MD-Tissue.
– Bar graphs displaying COL-I (B) protein levels analyzed densitometric scanning of immunoreactive bands in panel A. Data are expressed as mean ± SD for the 8 samples.
(C) Slot blot analysis for COL-I showing that COL-I expression originates from tenocytes X = mean.

FIG. 10

Bar graphs showing tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) gene expression after normalization on GAPDH mRNA levels.

– Data obtained from the 8 samples are expressed as mean ± SD. X = mean.



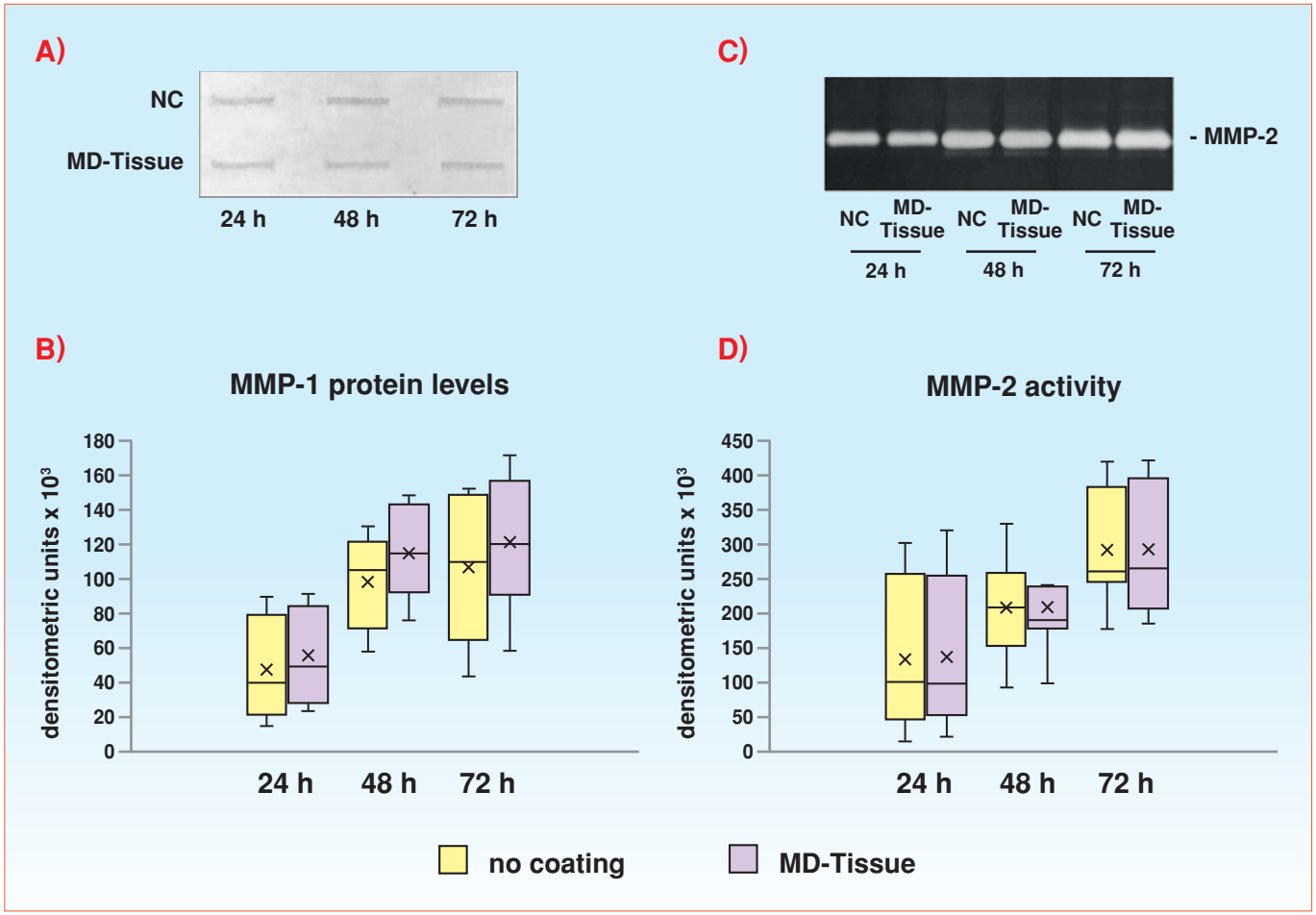


FIG. 11

Representative slot blot for matrix metalloproteinase-1 (MMP-1) levels (A) and representative SDS-zymography showing MMP-2 activity in serum-free cell supernatants of NC and MD-Tissue tenocytes.

– Bar graphs showing MMP-1 protein levels (C) and MMP-2 activity (D) after densitometric analysis of immunoreactive and lytic bands, respectively. Data obtained from the 8 samples are expressed as a % of densitometric units vs NC and are ± SD. NC: no coating. X = mean.

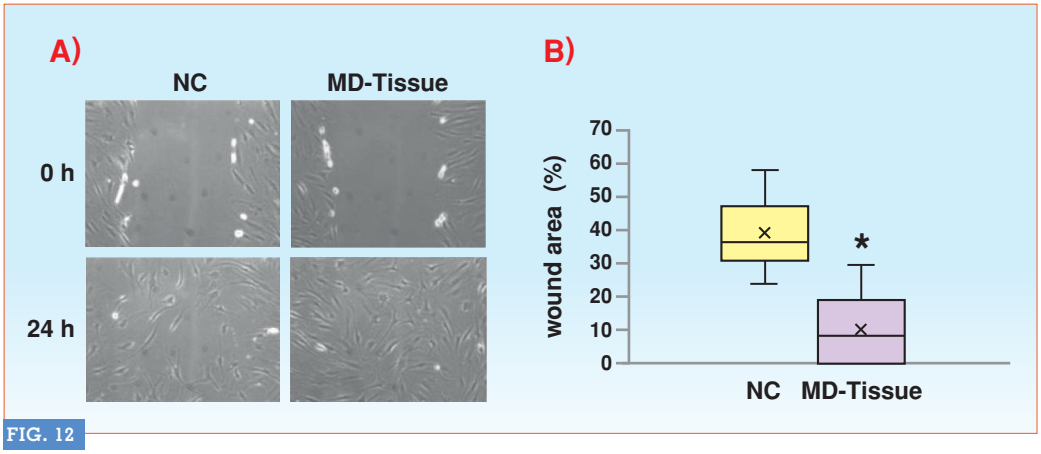


FIG. 12

(A) Representative micrographs showing wound healing assay in control tenocytes (NC) and tenocytes grown on MD-Tissue at 0 and 24 h after the scratch. Original magnification: 10x.
(B) Bar graphs showing the area of wound closure, expressed as a % of the area at 0 h, in cultured tenocytes in both experimental conditions 24 h after the scratch. * p <0.005 vs NC. X = mean.

Comments by the author
– These experimental data are crucial:
• **MD-Tissue INDUCES tenocyte proliferation and the neosynthesis of type I COL with a restoring and biological recovery effect, in addition to its bio-scaffold action.**

6-4 Increase in TIMPs - tissue inhibitors of the matrix metalloproteinases (FIG. 10)
One mechanism of action of the Collagen MDs consists in the **increase** of TIMP-1, the enzyme that inhibits the degradation of type I COL caused by MMP-1 and **not** in the direct **inhibition** of MMP-1, a degradation enzyme (FIG. 11).
– The increase in TIMP-1 makes it possible to control MMP-1 and MMP-2 by making them work in a physiological manner, and, therefore preventing their excess from triggering collagenolysis phenomena.

6-5 Tenocyte migration. Wound healing model (FIG. 12)
The tenocytes treated with MD-Tissue migrate towards the middle of the wound more effectively than the NC control tenocytes (A) with a consequent reduction in the size of the wound (B).

Author's notes – SUMMARY
This is the first basic (preclinical) trial on a Guna Collagen Medical Device **(MD-Tissue)**.
– Adding MD-Tissue to a culture of human tenocytes harvested surgically from healthy tendons produces **statistically significant** effects on:

A) Tenocyte proliferation
B) The neosynthesis of type I collagen by inhibiting its degradation
C) Tenocyte migration for wound repair.

• These 3 experimental findings are of **great importance** for understanding the molecular mechanisms of repair with MD-Tissue of damaged tendons (overuse, conditions of various aetiologies, ageing).

– MD-Tissue therefore fills a large therapeutic gap as, to date, there is no other device able to "do" what MD-Tissue "does".

The action of stem cells and PRP (Platelet-Rich Plasma) is akin to the action of MD-Tissue; however, they have generated conflicting results and have high (or very high) costs in addition to their operational complications.

Authors' conclusions
"Considered overall, these findings suggest that MD-Tissue acts as a **mechanical scaffold** that is able to induce an anabolic phenotype in tenocytes, thereby **favouring homeostasis** and **tendon repair** (…omissis). Our results suggest that MD-Tissue may represent a new therapeutic approach and stimulate the conduct of new clinical studies to analyse the effects of this product in the treatment of difficult conditions involving the tendons".

CONCLUSIONS

Two articles have been published regarding the therapeutic use of MD-Muscle in trigger points, the first (Staňa, 2016-17) in the treatment of piriformis syndrome, the second (Nitecka-Buchta et al., 2018), which is analytically presented in this paper, in the treatment of myofascial pain of the masseter muscle.

– Injection therapy of the myofascial trigger points is still, often and practically, the same as that proposed by Simons & Travell (1983), 35 years ago: local anaesthetic (Procaine, Lidocaine, Mepivacaine).
In addition, local injections with sterile saline solution, steroids and botulinum toxin have been, and still are, used.
Local anaesthetics (amongst others, Shah et al., 2015), steroids (amongst others, Fredberg, 2007), and botulinum toxin (amongst others, Davies & Barnes, 2000), when injected locally, present potentially **severe local** and **systemic side effects**.

As regards sterile saline solution, Nitecka-Buchta et al., 2018 (in this paper) obviously consider it a neutral control, rather than a control proper.
Indeed, sterile saline solution reduces pain by just one fifth (-20%) and reduces the EMGs voltages before/after therapy (2nd and last follow-up) by just one seventh (-14%).

– Considering the critical mass of clinical studies on the efficacy of Guna Collagen MDs, true evidence of their molecular mechanisms of action was lacking.

Having demonstrated that the Guna Collagen MDs are clinically efficacious, it remained to be seen how and why they were.

The response is provided by a very recent high-quality study, the result of a partnership between 3 highly-specialised Italian centres, that was recently published in Cells (see above).

In chronic painful rheumatic/joint diseases, each cycle of therapy with Guna Collagen Medical Devices (between 6 and 10 sessions) **must** be repeated at least once a year, as the biological halving time of COL is 360 days at the most (Olsen, 1983).

– In the light of the great many specific and substantiated clinical findings it can now be confidently stated that Guna Collagen Medical Devices are **1)** rapidly efficacious, **2)** safe, **3)** easy and straight-forward to apply and **4)** allow good adherence from both the patient's and the physician's standpoint.

Literature cited

A –Alemán A. *et al.* – Contribution of Leu and Hyp residues to antioxidant and ACE-inhibitory activities of peptide sequences isolated from squid gelatin hydrolysate. Food Chem 125: 3354-341; **2011**.
–Al-Rawi Z.S. *et al.* – Joint mobility among university students in Iraq. Br J Rheumatol. **1985**; 24(4): 326-31.
–Asserin J. *et al.* – The effect of oral collagen peptide supplementation on skin moisture and the dermal collagen network: evidence from an *ex vivo* model and randomized, placebo-controlled clinical trials. J. Cosmet Dermatol, **2015** Dec; 14(4): 291-301.
–Aviv A. *et al.* – The longevity gender gap: are telomeres the explanation? Sci Aging Knowledge Environ. **2005** Jun 8; 2005(23): pe16.

B –Beighton P. *et al.* – Hypermobility of Joints. 3rd ed. Springer Verlag, Berlin; **2012**.
–Bello A.E. & Oesser S. – Collagen hydrolysate for the treatment of osteoarthritis and other joint disorders: a review of the literature. Curr Med Res Opin., **2006** Nov; 22(11): 2221-32.
–Bhattacharjee A. *et al.* – Collagen structure: the Madras triple helix and the current scenario. IUBMB Life, **2005** Mar; 57(3):161-72.
–Bird H.A. – Special interest group for joint hypermobility. [British Society for Rheumatology meeting report]. Br J Rheumatol. **1993** Jan; 32(1):81.
–Biro F. *et al.* – The hypermobility syndrome. Pediatrics. **1983** Nov; 72: 702-6.
–Birrell F.N. *et al.* – High prevalence of joint laxity in West Africans. Br J Rheumatol. **1994** Jan;33(1):56-9.
–Brew K. *et al.* – Tissue inhibitors of metalloproteinases: evolution, structure and function. Biochim Biophys Acta. **2000**, 1477(1-2):267-83.
–Brew K. & Nagase H. – The tissue inhibitors of metalloproteinases (TIMPs): an ancient family with structural and functional diversity. Biochimica et Biophysica Acta **2010**; 1803(1): 55-71.
–Bridges A.J. *et al.* – Joint hypermobility in adults referred to rheumatology clinics. Ann Rheum Dis. **1992**; 52: 793-796.
–Bruyère O. *et al.* – Effect of collagen hydrolysate in articular pain: a 6-month randomized, double-blind, placebo-controlled study. Complement Ther Med., **2012** Jun; 20(3): 124-30.

C –Choi S.Y. *et al.* – Effects of collagen tripeptide supplement on skin properties: a prospective, randomized, controlled study. J. Cosmet Laser Ther **2014** Jun; 16(3): 132-7.
–Clark K.L. *et al.* – 24-Week study on the use of collagen hydrolysate as a dietary supplement in athletes with activity-related joint pain. Curr Med Res Opin. **2008** May; 24(5): 1485-96.
–Crowley D.C. *et al.* – Safety and efficacy of undenatured type II collagen in the treatment of osteoarthritis of the knee: a clinical trial. Int J Med Sci, **2009** Oct 9; 6(6): 312-321.
–Cutroneo K.R. *et al.* – Glucocorticoids and collagen synthesis: comparison of *in vivo* and cell culture studies. Collagen and related research, Vol I, issue 6, Nov **1981**; 557-568.

D –Davis E.C. & Barnes M.P. – Botulinum toxin and spasticity. J Neurol Neurosurg Psychiatry. Editorial. **2000** Aug; 69(2):143-7.

F –Fields G.B. – Synthesis and biological applications of collagen-model triple-helical peptides. Organic & Biomolecular Chemistry; **2010**, 8, 6 (1237).
–Fratzl P. – Collagen. Structure and Mechanics. Springer Science & Business Media, **2008**.
–Fredberg U. – Local corticosteroid injection in sport: review of literature and guidelines for treatment. Scand J Med Sci Sports. **2007** Jun;7(3):131-9.

G –Gartner L.P. & Hiatt J.L. – Extracellular matrix. *In:* Color Textbook of Histology (3rd ed.). Saunders **2007**: 73-5.
–Grahame R. – Clinical conundrum: how often, when and how does joint hypermobility lead to osteoarthritis? Br J Rheumatol **1989**; 28:320.
–Grahame R. & Jenkies J.M. – Joint hypermobility - asset or liability? A study of joint mobility in ballet dancers. Ann Rheum Dis. **1972**; 31:109-11.
–Grahame R.A. *et al.* – A proposed set of diagnostic criteria for the benign joint hypermobility syndrome. Br J Rheumatol, **1992**; 31:205.

H –Hand. A.R. & Ten Cate A.R. – Cytoskeleton, junction and fibroblasts. *In* Nanci A. (Ed.). Ten Cate's oral histology. Development, structure and function. India: Elsevier **2006**: 54-60.
–Heine H. – Manuale di Medicina Biologica. Regolazione di base e matrice extra-cellulare. Guna Ed., Milano; **2009**.
–Hexsel D. *et al.* – Oral supplementation with specific bioactive collagen peptides improves nail growth and reduces symptoms of brittle nails. J Cosmet Dermatol. **2017**; 16: 520-526.

I –Inoue N. *et al.* – Ingestion of bioactive collagen hydrolysates enhance facial skin moisture and elasticity and reduce facial ageing signs in a randomized double-blind placebo-controlled clinical study. J. Sci Food Agric **2016** Sep; 96(12): 4077-81.

J –Jonsson H. *et al.* – Hypermobility associated with osteoarthritis of the thumb base: a clinical and radiological subset of hand osteoarthritis. Ann Rheum Dis. **1996** Aug; 55(8): 540-3.

K –Koutroubakis I.E. *et al.* – Serum laminin and collagen IV in inflammatory bowel disease. J Clin Pathol. **2003** Nov; 56(11):817-20.
–Krum H. *et al.* – Relation of peripheral collagen markers to death and hospitalization in patients with heart failure and preserved ejection fraction re-

sults of the I-PRESERVE collagen substudy. Circulation: *Heart Failure* **2011**; 4(5), 561-568.
–Kumar V. & Gill K.D. – Basic Concepts in Clinical Biochemistry: A Practical Guide. Springer; **2018**.

L –Lacraz S. *et al.* – IL-10 inhibits metalloproteinase and stimulates TIMP-1 production in human mononuclear phagocytes. J Clin Invest. **1995** Nov; 96(5):2304-10.
–Lee S.K. *et al.* – Pressure ulcer healing with a concentrated, fortified, collagen protein hydrolysate supplement: a randomized controlled trial. Adv Skin Wound Care, **2006** Mar; 19(2): 92-6.
–Light N. & Champion A.E. – Characterization of muscle epimysium, perimysium and endomysium collagens. The Biochemical Journal **1984**; 219(3) 1017-26.
–Lotz M. & Guerne P.A. – Interleukin-6 induces the synthesis of tissue inhibitor of metalloproteinases-1/erythroid potentiating activity (TIMP-1/EPA). J Biol Chem. **1991** Feb 5;266(4): 2017-20.

M –Milani L. – Omeosiniatria e Trigger miofasciali: un'accoppiata vincente. Prima parte. La Med. Biol., **2003**/2; 31-41.
–Milani L. – Weihe e altri Punti. Tra Agopuntura e Omeopatia. Libro-Atlante. Guna Ed., **2004**.
–Milani L. – Un nuovo e raffinato trattamento iniettivo delle patologie algiche dell'Apparato locomotore. Le proprietà *bio-scaffold* del collagene e suo utilizzo clinico. La Med. Biol., **2010**/3; 3-15.
–Milani L. – I Collagen *Medical Devices* nel trattamento locale delle artroreumopatie algiche. Rassegna degli studi clinici e *clinical assessment* 2010-2012. La Med Biol., **2013**/2; 3-18.

N –Nakchum L. *et al.* – Preparation of squid skin collagen hydrolysate as an antihyaluronidase, antityrosinase, and antioxidant agent. Preparative Biochemistry and Biotechnology, **2016**; 46(2): 123-30.

O –Olsen B.R. – Collagen Biosynthesis. *In:* Hay E.D. (ed.): Cell Biology of Extracellular Matrix. 2nd ed. New York-London: Plenum; **1983**: 139-178.

P –Pavelka K. *et al.* – MD-Lumbar, MD-Muscle e MD-Neural nella terapia locale del dolore lombare. La Med Biol., **2012**/4; 13-17.
–Pavelka K. *et al.* – Chronic Low Back Pain: Current Pharmacotherapeutic Therapies and a New Biological Approach. Curr Med Chem. **2018**, 25:1-8.
–Proksch E. *et al.* – Oral intake of specific bioactive collagen peptides reduces skin wrinkles and increases dermal matrix synthesis. Skin Pharmacol Physiol, **2014**; 27(3): 113-9.

Q –Qin Z. *et al.* – Actin cytoskeleton assembly regulates collagen production via TGF-β type II receptor in human skin fibroblasts. J Cell Mol Med. **2018** Sep; 22(9):4085-4096.

R –Reichenstein M. *et al.* – ACTH induces TIMP-1 expression and inhibits collagenase in adrenal cortex cells. Molecular and Cellular Endocrinology **2004** Feb; 27, 215(1-2):109-14.

S –Saladin K.S. – Anatomy and Physiology: The Unity of Form and Function. New York, NY: McGraw-Hill, **2012**.
–Schmidt R.E. & Burkhardt H.E. – Bindegewebe Siegenthaler W. (Hrsg.): Klinische Pathophysiologie. 8. Aufl. Stuttgart New York: Thieme; **2001**: 978-1018.
–Schmitt E. *et al.* – Switching from an induced fit to a lock and key mechanism in an aminoacyl-tRNA synthetase with modified specificity. J Mol Biol. **2009** Dec 18; 394(5): 843-851.
–Schunk M. *et al.* – Dietary Supplementation with Specific Collagen Peptides Has a Body Mass Index-Dependent Beneficial Effect on Cellulite Morphology. J Med Food. **2015** Dec 1; 18(12): 1340-8.
–Shah J.P. *et al.* – Myofascial Trigger Points Then and Now: A Historical and Scientific Perspective. PM&R: the journal of injury, function, and rehabilitation. **2015** Jul; 7(7):746-761.
–Shoulders M.D. & Raines R.T. – Collagen structure and stability. Annu Rev Biochem. **2009**; 78:929-58.
–Silverman S. *et al.* – Survey of joint mobility and *in vivo* skin elasticity in London schoolchildren. Ann Rheum Dis. **1970**; 32:352-7.
–Simons D.G. & Travell J.G. – Myofascial Pain Dysfunction: The Trigger Point Manual. 1st Ed. Lippincott Williams & Wilkins, **1983**.
–Söderhäll C. *et al.* – Variants in a novel epidermal collagen gene (COL29A1) are associated with atopic dermatitis. PLoS Biol. 5:e242; **2007**.
–Song H. *et al.* – The effect of collagen hydrolysates from silver carp *(Hypophthalmichthys molitrix)* skin on UV-induced photo-ageing in mice: molecular weight affects skin repair. Food Funct 8(4): 1538-46; **2017**.
–Staňa J. – 3 years in Luhačovice Spa with Collagen Medical Devices injections in the treatment of piriformis syndrome. Physiological Regulating Medicine, **2016-17**; 19-20.

T –Tanrikulu I.C. *et al.* – Peptide tessellation yields micrometre-scale collagen triple helices. Nat Chem. **2016** Nov; 8(11):1008-1014.
–Tomasek J.J. *et al.* – Collagen modulates cell shape and cytoskeleton of embryonic corneal and fibroma fibroblasts: distribution of actin, alpha-actinin, and myosin. Dev Biol. **1982** Jul; 92(1):107-22.

V –Van Vijven J.P. *et al.* – Symptomatic and chondroprotective treatment with collagen derivatives in osteoarthritis: a systematic review. Osteoarthritis Cartilage. **2012** Aug; 20(8):809-21.

W –Wu L. – Collagens at a glance. Top. Curr. Biol., **2011**; 147:139-178.

NOTES

Figures 4, 5, 7, 8, 9, 10, 11, 12 and Tables 2, 3, 4 have been graphically recreated from the original articles.
Figure 3 is from (detail): Lanza B., Azzaroli Puccetti M.L., Poggesi M., Martelli A. – Le cere anatomiche della Specola. Arnaud Ed., Firenze, 1979. The anatomical positioning of TPs is by the author.
The captions of Figure p. 3, Figures 1, 2, 3 and 6 are by the author.
Figure 2 and Table 1 are by the author.

The author would like to thank the Internet sites from which are taken the following Figures:

Fig. p. 3
http://herenow250.rutgers.edu/gallery/category/arts?page=9
Fig. 1
https://c1.staticflickr.com/2/1593/25648507056_2726320d17_b.jpg
Fig. 1 (enlargement in the top left corner)
http://www.biology-pages.info/C/Collagen.gif
Fig. 6
https://i.ytimg.com/vi/KyoAhyN97ZM/hqdefault.jpg
Fig. 6 (enlargement in the top left corner)
https://www.sciencephoto.com/media/80294/view/fibroblasts-sem

author

Prof. Leonello Milani, MD, PhD
– Scientific director of "La Medicina Biologica" and "Physiological Regulating Medicine"
Via Palmanova, 71
I – 20132 Milano